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Further Experiments on the Chemical Treatment of Soil infected with *Heterodera schachtii*.

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Preliminary experiments were carried out during 1935, on the possible application of certain chemical substances to the problem of controlling "potato-sickness." The results were published in two papers by Hurst & Triffitt (1935, i, ii) and it was shown that calcium cyanamide and ferric oxide were deserving of further trials on a larger scale. Field experiments were devised and carried out during 1936 in various parts of the country, and the results will be communicated when these are available. In the meantime the small-scale experiments were continued and are described below.

Calcium Cyanamide.

In the previous experiments a series of pots was prepared in which soil infested with cysts of *Heterodera schachtii* was mixed with varying amounts of calcium cyanamide, equivalent to dressings of 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 cwt. per acre. In addition a series of pots containing urea, a decomposition product of calcium cyanamide which in sufficient amount was also toxic to *H. schachtii* larvae, was prepared, the nitrogen contents being the same as in the cyanamide series. "Seed" potatoes were planted and at the end of ten weeks the pots were examined,

in the manner previously described, for the counting of cysts newly formed on the roots. The average results are shown in Table I.

When these cyst counts had been made, the soil in each case was thoroughly mixed and returned to the pot, for the purpose of growing potatoes again in 1936. None of the newly formed cysts was removed, so that subsequent results would more nearly conform to those obtained in field experiments.

TABLE I.
(1935 Pot Experiments.)

Calcium Cyanamide Series		Urea Series	
Amount of dressing (cwt. per acre)	No. of cysts on roots	Amount of dressing (cwt. per acre)	No. of cysts on roots
0 (Controls)	Approx. 190	0 (Controls)	Approx. 190
10	,, 100	4·5	,, 100
15	,, 150	6·7	,, 100
20	29	9	,, 150
30	2	13·5	,, 150
40	14	18	,, 100
50	Nil	22·5	27
60	3	27	6
70	Nil	31·5	Nil
80	Nil	36	1
90	Nil	40·5	1
100	Nil	45	Nil

A little of the soil was removed from each pot and cysts were separated for hatching experiments. These gave widely varying results for the urea series, but the general conclusion was that whilst cysts from soil dressed with 20 cwt. per acre and upwards of cyanamide showed little or no tendency to liberate larvae when subjected to the stimulus of potato root-excretion, those from every member of the urea series hatched freely, often in greater numbers than those from the control pots.

Potatoes were again planted in the pots in 1936, and also in another cyanamide series which was not mentioned in the previous communication. When the experiments were commenced in 1935 it was thought highly probable that the considerable excess of nitrogen in the heavier cyanamide dressings would so upset the nitrogen-potash-phosphate balance that growth of the potato plant would be very much retarded. For this reason an extra pot of each of the series was prepared and planted with cabbage. Such a plant, with its large area of leafage, would be likely to remove much of the excess nitrogen from the soil. Moreover, the absence of the potato plant would allow the cyanamide a longer period, if such were found desirable, in which to exercise a lethal action on the eggs contained in the cysts.

The expectation of retardation in potato plant growth in the heavier cyanamide dressings was not, in fact, realised in the pot experiments, but subsequent experience has shown that it may be much in evidence in field

TABLE II.
(1936 Pot Experiments.)

Calcium Cyanamide Series			Urea Series	
Amount of dressing (cwt. per acre)	No. of cysts on roots		Amount of dressing (cwt. per acre)	No. of cysts on roots. Potato following potato
	Potato following potato	Potato following cabbage		
0 (Controls)	135	—	0 (Controls)	135
10	38	21	4·5	24
15	19	20	6·7	140
20	15	Nil	9	94
30	4	4	13·5	51
40	2	6	18	113
50	Nil	Nil	22·5	82
60	2	2	27	16
70	Nil	Nil	31·5	20
80	Nil	Nil	36	61
90	Nil	Nil	40·5	Nil
100	Nil	Nil	45	Nil

experiments. The cabbage series, therefore, presents an interesting comparison since, in the absence of the host plant, the lethal action of cyanamide was confined to the dormant larvae contained in the cysts, and did not include the possibility of action on hatched larvae during their passage through the soil.

In the cyanamide series the growth response of the potato plants in the soils containing dressings of over 20 cwt. per acre was almost as conspicuous as in the previous year. In the urea series, on the other hand, the response was much less marked than previously, and it appeared probable that most of the residual nitrogen had been removed in the leachings. After about two months of potato growth the pots were emptied and the numbers of newly formed cysts counted. The results are given in Table II.

It appears probable that urea, except possibly in the heaviest dressings used, had not been lethal to the contents of many of the cysts, and that the results obtained from the first growth of potatoes in 1935 must, in part, be attributed to a strong, but temporary, retarding action on the hatching of larvae. Calcium cyanamide, however, fully maintained its earlier promise, and the results for the series following cabbage were for practical purposes the same as for the series in which potatoes were grown a second time. The importance of these results, with a view to field experiments, will be discussed later.

Ferric Oxide.

During 1935 experiments were carried out at the Institute's Field Station, St. Albans, on a small plot of land ($\frac{1}{20}$ acre) infested with *H. schachtii* cysts. Dressings of various chemicals were applied, and it was found that the yield of potatoes from each row of an area treated with ferric oxide was uniformly high. To test the possibility that this might have happened by chance, potatoes were again planted in 1936 and five rows towards each end of the plot were treated with ferric oxide (B.D.H. precipitated). In view of previous successive years' experience, the areas treated were not regarded as more fertile than the remainder of the plot. On the day previous to the planting of Duke-of-York "seed" potatoes, an artificial mixture containing ammonium sulphate, superphosphate and potash in the proportion 3 : 4 : 3 was applied at the rate of ten cwt. per

acre. Ferric oxide was spread along ten of the rows at the time of planting and in the manner formerly described.

The plants on the treated areas, in general, developed more rapidly than the controls and were superior in appearance throughout the season. The yields from individual rows, neglecting two at each end, are recorded in Table III.

The results, following those of the previous year, are suggestive that ferric oxide had a real effect in increasing the yield on this type of soil. To test whether any effect on yield would be observed in the absence of *H. schachtii* infestation, a nearby plot with soil of similar texture but free from eelworm infection, was submitted to the following treatment :—

TABLE III.
(Yields from *H. schachtii* plot).

Ferric Oxide		Controls			
Row No.	Yield (lbs.)	Row No.	Yield (lbs.)	Row No.	Yield (lbs.)
3	12	8	10	19	13½
4	18	9	12½	20	16
5	20½	10	15	21	14½
6	20	11	12	22	13
7	18	12	10	23	17
30	14	13	10	24	12½
31	20	14	11½	25	11½
32	23	15	12½	26	13½
33	21½	16	17	27	13½
34	22½	17	12	28	13
—	—	18	15	29	12½
Average 19 lbs.		Average 13 lbs.			

One half of the plot was treated with artificials, the other half remaining untreated. Ferric oxide was applied to some of the rows in each half and the same variety of "seed" potatoes (Duke-of-York) was planted. In this experiment, the plants on the treated areas appeared rather poorer than the controls in the early stages of growth, but no difference was observed later. The yields from individual rows are shown in Table IV.

It will be seen that the yields from the ferric oxide rows were not appreciably different from the control yields, irrespective of whether the land had previously been treated with artificials.

TABLE IV.

(Yields from non-infected plot).

	Ferric Oxide		Controls	
	Row No.	Yield (lbs.)	Row No.	Yield (lbs.)
No Artificials	8	20½	12	20
	9	21	13	20½
	10	21	14	23
	11	23	15	21½
	16	25	20	26
	17	20½	21	21
	18	28	—	—
	19	28	—	—
Plus Artificials	22	29	24	20
	23	27½	25	27
	28	20½	26	27
	29	26½	27	18
	30	22	32	24
	31	18	33	20
	36	22	34	22½
	37	22½	35	25

Discussion.

Ferric Oxide.—The results of the experiments with ferric oxide suggest that on this particular piece of infected land an effect was produced which led to an increase in yield. It should, however, be pointed out that larger field experiments on entirely different soil were carried out during 1936 in various parts of the country, and in no case were the beneficial results described above obtained. Details of these results, with the effect of ferric oxide treatment on new cyst formation, will be presented in separate communications.

Calcium Cyanamide.—The results of pot experiments with calcium cyanamide have interesting applications to field experiments. The difficulty, as previously emphasised, is to procure intimate mixing of the chemical with soil. In field experiments during 1936 a rototiller was used on land which received dressings of cyanamide up to 40 cwt. per acre. In some cases the soil was of a light sandy nature and appeared very suitable for even mixing with the dressings. But, although results of much promise were recorded, in no case did the effective lethal action of the cyanamide approach that obtained in the pot experiments. This might be expected, since in the latter experiments the soil was air-dried, powdered and sieved, and then mixed very thoroughly with the cyanamide by hand.

During the coming season the effect of even larger dressings than 40 cwts. per acre will be tested in field scale experiments. In such cases it would probably be unprofitable to attempt to grow potatoes until some time had elapsed. The results described in this paper do show, however, that calcium cyanamide is lethal to the cyst contents and that the extent of its action is independent of the nature of the plant grown after its application. If therefore it is found possible to grow a green crop before potatoes are again planted, the reduction in the large excess of soil nitrogen which would be obtained might prove eventually to be very beneficial.

There is no reason to suppose that calcium cyanamide may not, with equal promise of success, be applied to soil infested with other strains of *H. schachtii*, and field experiments on oats have been planned for the coming season.

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Field Experiments in Lincolnshire on the Chemical Treatment of Soil infected with *Heterodera schachtii*.

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This communication is concerned with the field trials of substances which in previous small-scale experiments had shown promise of controlling " potato-sickness " when they were mixed with infected soil.

It was shown by Hurst & Triffitt (1935, i) that calcium cyanamide in sufficient quantity was lethal both to hatched *H. schachtii* larvae and to the eggs contained in the cysts. A detailed series of pot experiments led to the conclusion that a dressing equivalent to at least one ton per acre was necessary for the limitation of newly-formed cysts to a very small number. It was realised that in a field experiment, with the greater difficulties of obtaining intimate mixing of the chemical with soil, a much larger dressing might be required to produce equivalent results. On the other hand, it appeared likely that too large a dressing in the field might, by its effect on the nitrogen-phosphate-potash balance, have a serious retarding effect on the growth of the potato plant. It was decided to compromise between these considerations as far as seemed profitable without previous knowledge, and to apply cyanamide at the rate of 30 cwt. per acre.

It was also shown by Hurst & Triffitt (1935, ii), that in a small field-scale experiment on infected land at the Institute's farm at St. Albans, the application of ferric oxide to the surface of the furrows at the time of planting led to a greater yield of potatoes. Laboratory experiments had suggested that this substance tended to neutralise the principle of potato root-excretion which induces the hatching of *H. schachtii* larvae, but that its effect was limited to the first few days of plant growth. The amount of ferric oxide which gave these results was approximately 6 cwt. per acre, and it was decided to use double this quantity in the present experiments.

Field Experiments.

The experiments were carried out during 1936 on "potato-sick" land of the Agricultural Institute, Kirton, Lincs., by the kind permission of the Principal, Mr. J. C. Wallace, and with the co-operation of Mr. J. Wood, A.R.C.S. Mr. Wood's personal supervision of the agricultural operations and his advice on numerous questions which arose during the progress of the work were of great help and were much appreciated.

The piece of land available was rectangular and measured 162 by 78 feet, *i.e.*, approximately $\frac{3}{10}$ acre. The soil was a light silt, with a faintly alkaline reaction. Potatoes were grown on this land in 1933, since when trefoil and grasses had been grown. One-third of the area was ploughed up in the spring of 1935 and potatoes were planted, but the severe frost in May of that year killed the plants. The arrangement of the present plots was such that the possible residual effects of previous treatment were evenly distributed. The area was divided into 18 plots, each measuring 78 by 9 feet, and as the rows of potatoes were spaced at intervals of 27 inches, each plot contained four rows. The plots were arranged to represent two 3 × 3 Latin squares (Fig. 1), the treatments being A (Control), B (Calcium cyanamide), and C (Ferric oxide).

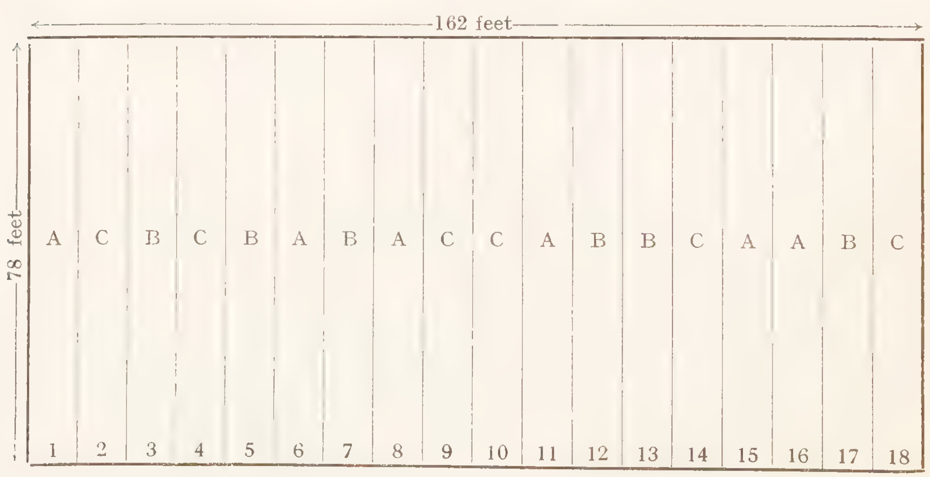

Fig. I.

Arrangement of Plots (to scale).

A = Control.
B = Calcium cyanamide.
C = Ferric oxide.

The calcium cyanamide used was the commercial oiled product, for which the following typical analysis was supplied by the manufacturers :—

Calcium cyanamide	56·0
Dicyanodiamide	0·1
Calcium carbonate	6·0
Calcium sulphide	1·1
Calcium sulphate	inappreciable
Calcium carbide	trace
Free Lime	11·8
Calcium otherwise combined (calc. as CaO) ...	4·5
Free Carbon	10·9
Iron oxide and Alumina	2·3
Silica	1·1
Oil	1·6
Moisture	0·2
Undetermined	4·4
	100·0
Total N_2	20·7%
Total Lime (calc. as CaO)	60·2%

This was applied to the plots marked " B," on April 8, at the rate of 30 cwt. per acre, and was incorporated with the soil by means of a roto-tiller, used twice on each plot. A period of about one month was allowed to elapse before the planting of potatoes, to permit of the decomposition of the cyanamide beyond the stages at which it might be toxic to the growing plant. The soil-moisture at the time of application of the cyanamide was 22·0 per cent. and at the time of planting on May 9 was 18·1%. It appeared from previous laboratory experiments [Hurst & Triffitt (1935, i)] that there was some correlation between degree of soil-moisture and toxicity of cyanamide to *H. schachtii*. The results of these experiments suggested that for maximum lethal action of the chemical, the soil should be drier than the above. Artificials were applied to the plots on May 8, the day before planting. The mixture used was one of the customary Lincolnshire dressings for main-crop potatoes, recommended by the Kirton Agricultural Institute, and contained 8·3% ammonia, 13·3% soluble phosphate, 11·1% potash, and 6·0% insoluble phosphate.

This was applied at the rate of 16 cwt. per acre. In view of the heavy dressing of nitrogen which the cyanamide plots had received, it would probably have been desirable to omit sulphate of ammonia from the artificial mixture. This substance was, however, included since it was equally desirable to keep the dressing of cyanamide as the only difference in treatment between these plots and the controls. On the following day potatoes were planted, the rows being 27 inches apart and the setts 16 inches apart. The " seed " used was once-grown Majestic. At the time of planting and while the potatoes were exposed in the rows, ferric oxide at the rate of 13 cwt. per acre was applied to the furrows of the plots marked " C," the procedure adopted being identical with that described by Hurst & Triffitt (1935, ii). In place of the relatively costly B.D.H. precipitated ferric oxide which had previously been used, a high quality of commercial " levigated " ferric oxide was substituted, and there appeared no reason to anticipate any difference in the action of these two chemicals.

We are indebted to Mr. Wood for observations made during the growing season. The summer of 1936 was abnormally wet and this may have contributed to the rapid growth and healthy appearance of plants on the control plots. These were, in fact, the best seen for several years on infested land at Kirton. The plants on the ferric oxide plots were, on the whole, not very different in size and appearance from the controls. They appeared to have a slight advantage in vigour, but were somewhat poorer in colour. Those on the cyanamide plots were, however, conspicuous for the dark green colour of the leaves and for the marked retardation in growth during the early period. After about two months a rapid increase in rate of growth was observed, and towards the end of the season they were larger and stronger than those on adjacent plots. The darkness in colour of leaf remained throughout the growing period.

Yellowing of the controls commenced about the end of June, followed closely by plants on the ferric oxide plots. At this stage " blight " attack was feared and the plants were treated on several occasions during July and August with a 15 per cent. copper fungicide applied at the rate of 10 to 20 lbs. per acre. By this means the crop was kept practically free from attack.

The plots dressed with cyanamide were conspicuously free from weeds until late in the season.

The crop was " lifted " on October 13 by the use of a spinner followed by a harrow, and the yields of " ware " and " chats " from each row were weighed. " Seed " was not separated from the " chats " since it was not customary to retain " twice-grown seed."

Yields.

It was pre-arranged that the yields from the centre two rows only, of each plot, should be considered in the comparison of treatment-yields, although in point of fact the comparison of total yields did not differ significantly from this. The yields of " ware " and " chats " from the centre two rows of each plot are shown in Fig. 2, where the representation of plots is in the Latin square form.

Plots 1 to 9.

A 180 (35)	C 178 (39)	B 233 (23)
C 134 (38)	B 227 (30)	A 147 (33)
B 155 (38)	A 123 (42)	C 144 (38)

Plots 10 to 18.

C 76 (36)	A 124 (31)	B 208 (22)
B 232 (19)	C 84 (48)	A 133 (40)
A 148 (34)	B 147 (19)	C 204 (30)

Fig. II.

Yield in lbs. of " ware " and (in parentheses) " chats " from centre two rows of each plot.

A = Control.
B = Calcium cyanamide.
C = Ferric oxide.

That the increase in yield of " ware " obtained from the cyanamide plots is a real one, is strongly suggested by the accompanying decrease in yield of " chats." For the statistical examination of the results, the yield of " ware " only is considered. Taking the two Latin squares together, and using Fisher's method of the analysis of variance, there are in all 17 degrees of freedom. These are made up as follows :—rows 4, columns 4, squares 1, treatments 2, and errors 6. The standard error calculated from these yields is shown in Table I.

TABLE I.

	A (Control)	B (Cyanamide)	C (Ferric oxide)	Standard Error
Yield of "ware" in lbs. (centre two rows of plots)	855	1,202	820	91·4
Tons per acre	7·90	11·10	7·57	0·84

The increase in yield of cyanamide plots compared with control plots is more than three times the standard error and is therefore significant. If Fisher's *t* test is applied, it is found that this difference in yield might have occurred by chance, less than 4 times in 100 trials.

If we consider the results from the whole four rows of each plot, thereby doubling the area of the experiment, the difference between yields from the cyanamide plots and control plots is still significant (Table II.).

TABLE II.

	A (Control)	B (Cyanamide)	C (Ferric oxide)	Standard Error
Total yield of "ware" (lbs.)	1,765	2,335	1,674	159·6
Tons per acre	8·15	10·78	7·73	0·74

The potatoes from the cyanamide plots showed a slight darkening on boiling, but not in sufficient amount to render them unsaleable. The potatoes will be tested again, after storage. The yield from the ferric oxide plots was not, in bulk, different in appearance from the control yield. Closer examination revealed occasional tubers to which some of the powder adhered. After peeling, these were indistinguishable from the controls both in appearance and in cooking quality.

It should be emphasised, however, that these experiments refer specifically to one type of soil, and it does not follow without trial that similar results, either in growth-response of the potato plant or in appearance and quality of the yield, would be obtained on other soil-types.

Soil Reaction.

pH measurements were carried out on composite samples of soil for each treatment, taken before and after the crop in the cases of control and ferric oxide plots, and in the case of cyanamide plots, before the chemical was applied, one month later at the time of planting, and again after the crop. The results are shown in Table III, from which it will be seen that although cyanamide caused an appreciable increase in alkalinity in the period preceding planting, at the end of the season the soil of each treatment showed approximately the same pH value.

TABLE III.

pH (Quinhydrone electrode).

	A (Control)	B (Cyanamide)	C (Ferric oxide)
Before treatment ...	—	7·4	—
At time of planting ...	7·6	8·0	7·5
After crop	7·3	7·3	7·3

Cyst Counts.

Soil samples, to a depth of nine inches, were taken by means of a cylindrical soil-sampler. It was found possible to have a hinge fitted along the length of the cylinder of the sampler, enabling it to be opened out after it had been filled with soil. Emptying of the sampler was very much facilitated by this device. A bulk sample of soil, consisting of at least six individual samples, was collected from each plot before the treatments were applied, and again after the crop had been "lifted." The bulk samples were air-dried, ground and sieved. For cyst counts, 50-gram portions of the well-mixed soil were weighed and the cysts separated by the customary flotation method. The filter papers which received the cysts and accompanying débris were allowed to dry. The material was then transferred, in three separate portions, to a sheet of thin Bristol Board. From this the larger free cysts were rolled on to white paper and counted. The débris from each portion, with many adhering and enmeshed cysts, was transferred to a narrow trough of Bristol Board, and the cysts counted under the binocular microscope.

The results of cyst counts from individual plots showed considerable variation, and it soon became apparent that a very large number of counts would, in the aggregate, be necessary if accurate comparisons were to be made between the cyst content of the soil of each plot before and after the crop. For this reason it was decided to mix equal quantities of the soil samples from similarly treated plots. There were thus six composite samples representing control plots, cyanamide plots, and ferric oxide plots, before and after the crop. The results of cyst counts from these composite samples are shown in Table IV.

TABLE IV.

Cyst counts from 50-gram soil samples.

	A (Control)		B (Calcium Cyanamide)		C (Ferric Oxide)	
	Before crop	After crop	Before crop	After crop	Before crop	After crop
No. of samples counted ...	15	15	27	27	15	15
Mean No. of cysts per sample	91·1	128·0	97·6	105·4	82·1	120·9
σ (Standard deviation of a single count) ...	±9·8	±14·1	±10·5	±11·6	±6·6	±9·7
σ_m (Standard error of mean)	±2·5	±3·6	±2·0	±2·2	±1·7	±2·5
Average increase per sample after crop	+36·9		+7·8		+38·8	

It should be pointed out that, in our experience, counts obtained from soil samples taken immediately after the crop are in many cases not absolutely comparable with those obtained from samples taken before the crop. The newly formed cysts may not be distributed evenly in the soil by the particular process adopted for lifting the potatoes. In the present case the use of a spinner was a very efficient method of mixing the soil, although it had the disadvantage of throwing a little of the soil from one plot to the next. The figures recorded in Table IV are, in any case, of much interest from a comparative point of view. It will be seen that the increases in cyst counts after the growth of potatoes on the control and ferric oxide plots are substantially the same, the difference being well within the limit

of experimental error. This was to be expected since, as was shown by Hurst & Triffitt (1935, ii), the possible retarding effect of ferric oxide on the hatching of larvae would be confined to the very earliest period of plant growth. Moreover, in the abnormal weather conditions experienced during 1936, potato-sickness was not visibly in evidence on any of the plots, and in these circumstances it is possible that results differing from the control might not be expected to follow the use of ferric oxide. In view of the much greater promise of cyanamide treatment, the ferric oxide plots are, as will be discussed later, being used for a further cyanamide trial.

The increase in the number of cysts after cyanamide treatment is small compared with other plots. It is, nevertheless, a significant increase. For the statistical comparison of the means of two series, the method described by Fisher (1936) may be used. This involves calculation of the statistic t. When, as in the present case, the number of individuals in each of the two series is the same, the required equation is simplified and becomes

$$t = (\bar{x} - \bar{x}^1)\sqrt{\frac{n+1}{\sigma_1^2 + \sigma_2^2}} ,$$

where, in the cyanamide counts,

$\bar{x}-\bar{x}^1$ = difference between means = 7·8
$n+1$ = nr. of counts in each series = 27
σ_1 = standard deviation in one series = 10·5
σ_2 = standard deviation in the other series = 11·6.

Substituting these figures we obtain $t = 2{\cdot}6$, and from Fisher's Table of t we obtain a probability of less than 1 per cent. that the cyanamide counts before and after the crop are members of the same series. The above calculation has been presented in some detail for reasons which follow :

Firstly, by substituting in the equation a value for t which is just statistically significant (giving a probability of 5 per cent.), and assuming that the numbers of counts and the standard deviations remain the same, we find that the difference between the means before and after the crop would be approximately 6·0. Secondly, it can be calculated that with the same standard deviations it would require a minimum of 16 counts before and 16 after the crop to make the difference between the means of 7·8 a significant one. If we assume that, as in the present experiments,

a potato plant occupies a volume of soil of dimensions 27 × 16 × 9 c. ins., the weight of this soil in the air-dry condition would be very approximately 65,000 grams. An increase of one cyst in a 50-gram soil sample would therefore correspond with an increase of about 1,300 cysts per potato plant. It must therefore be concluded that, if the figures recorded in Table IV are accurate, they indicate :

1. An average increase per potato plant on the control plots of about 48,000 cysts, on the ferric oxide plots of about 50,000 cysts, and on the cyanamide plots of about 10,000 cysts. These figures would, moreover, be minimum ones, since it has been assumed that none of the cysts present before planting had been destroyed by cultivation operations.

2. With the accuracy of counting recorded for the cyanamide samples, with 27 counts before and 27 after the crop, an increase of about 8,000 cysts per plant would be necessary for such increase to be shown significant.

3. With the increase found of about 10,000 cysts per potato plant on the cyanamide plots, a minimum of 16 counts, of the recorded accuracy, before and after the crop would be required to establish the significance of this increase.

Cyst Hatchings.

Samples of soil were obtained from the cyanamide plots at the time potatoes were planted, that is, about one month after the dressing of cyanamide was applied to the soil. Cysts were separated from these samples and placed in petri dishes containing potato root-excretion. In all cases some hatching of larvae took place, but the number was smaller than in parallel control experiments. These results, however, merely anticipated the observed fact that fewer larvae developed in the plants on the cyanamide plots. In these and other field experiments where heavy dressings of cyanamide up to 40 cwt. per acre have been applied, new cysts were usually seen when one of the growing plants was removed from the soil and the roots examined. Removal of a control plant at the same time invariably revealed a heavier infection, the difference in some cases being a striking one.

Since cyanamide appears to have been lethal to some of the cyst contents, the addition of a number of new cysts as a result of plant growth does not necessarily mean that the plots have become more heavily infected than they were before cyanamide was applied. The most satisfactory laboratory

experiment to test this, is to compare the degree of hatching from cysts obtained before cyanamide was applied with that from cysts obtained after the crop. The true comparison in such an experiment is between the cysts contained in equal quantities of soil. Thus, referring to the averages in Table IV, the 97·6 cysts before the crop are comparable with the 105·4 cysts after the crop.

These experiments will of necessity be deferred until the spring, since it is seldom possible to obtain reliable results from hatching experiments carried out during the winter months.

Discussion.

It may be said that, for this type of soil at least, the use of calcium cyanamide in the control of "potato-sickness" shows distinct promise. On the basis of these experiments the cost of the treatment was more than compensated for by the additional yield of potatoes, while the addition of new cysts to the soil was relatively small. The results recorded in this paper give no evidence to decide the extent to which the increase in yield was due to protection of the plant from eelworm invasion, or to the action of the cyanamide purely as a fertiliser. This question is, however, being examined in a field experiment elsewhere, by comparing the effects of equivalent dressings of calcium cyanamide and ammonium sulphate plus lime on infected soil.

How far the addition of new cysts to the cyanamide plots might be effective in checking plant growth will be determined during the coming season, when potatoes (second-earlies) will be grown on these plots.

It is not thought that the conditions of these experiments, with the absence of the customary severe symptoms of "potato-sickness" on the control plants, were entirely suitable to decide whether ferric oxide could have a beneficial effect on the yield from "potato-sick" land. In view, however, of the large increase in the number of newly-formed cysts, it was considered that it might be profitable to convert these plots into a cyanamide experiment in which the chemical was applied some months before potatoes were planted. It was decided to apply cyanamide at the rate of 40 cwt. per acre to the plots previously treated with ferric oxide. Some delay was caused by weather conditions and the chemical was applied on December 22. The plots receiving it were roto-tilled twice and, for uniformity, the remainder of the plots were roto-tilled at

the same time. It is thought that, particularly after roto-tilling, the possibility of the ferric oxide having any residual effect is remote.

It is hoped, also, to carry out chemical analyses on the soil samples obtained after the crop, chiefly with regard to the nitrogen-phosphate-potash ratio. These might suggest the kind of artificial mixture which would be most suitable for use on the cyanamide plots when potatoes are again planted.

Acknowledgments.

The authors wish to express their indebtedness to Mr. J. C. Wallace for allowing these experiments to be carried out on the land of the Kirton Agricultural Institute, and to Mr. J. Wood for his co-operation throughout the work. Thanks are due to Dr. F. W. Handley for carrying out soil-moisture determinations.

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Potato Eelworm (*Heterodera schachtii*): Further investigations.

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The investigations described in this paper are in part a continuation of those described by Carroll and McMahon in a previous paper published in 1935. The previous paper demonstrated the fact that the root excretion of potatoes growing in recently sterilized soil does not possess the power of inducing the hatching of eelworm eggs in the normal manner. It was shown that when eelworm cysts were placed in soil leaching (containing potato root excretion) obtained from pots of very recently sterilized soil growing potatoes practically no hatch of the eggs in these cysts took place until after the lapse of about thirty days. It was further indicated that this interval of time which elapses before hatching commences diminishes according as the period between time of sterilization of the soil and time of obtaining soil leaching for hatching experiment increases.

These hatching experiments, and also an extensive series of pot experiments, clearly demonstrated that where hatching of eelworm eggs in the soil is delayed potato plants growing in that soil get a chance of making some growth and establishing a good root system before they are attacked by newly hatched larvae, and that the onset of eelworm attack only when a good root system has been established does not seem to have much effect on the further growth or productivity of the plants. It was therefore suggested that whenever potato plants growing in the field happen to make some growth and establish a good root system before normal hatching of eelworm eggs in the soil commences these plants will show little or no sign of potato sickness during the subsequent period of their growth and will produce a good yield in spite of belated eelworm attack.

Experiments carried out since the previous paper was published confirm the results obtained in the earlier experiments (with one exception which will be mentioned later). It has been considered advisable to present a summary of some of these experiments.

Hatching Experiment in the Laboratory.

This experiment was carried out in 1935 (commenced on 24th April) using specially selected large cysts (from roots of potatoes grown in 1934), as was done in previous experiments. Eighty cysts (eight lots of ten cysts each) were used for each different root extract (soil leaching). The experiment was carried out in exactly the same manner as described in the previous paper, and the following tests were made :—

A—Hatching in leaching from unsterilized soil growing potato.

B— ,, ,, ,, soil sterilized previous Dec. growing potato.

C— ,, ,, ,, ,, ,, ,, Feb. growing potato.

D— ,, ,, ,, ,, ,, 10th April growing potato.

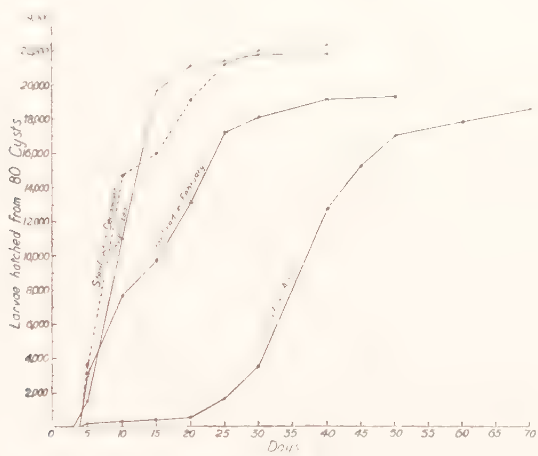
A summary of the result of this experiment is tabulated (Table I) and figured graphically as was done in the previous paper.

Table I

Soil leaching	Total hatch from 80 cysts after lapse of				
	5 days	10 days	15 days	20 days	25 days
A (unsterilized) ...	1,500	11,000	19,700	21,200	21,500
B (sterilized Dec.) ...	3,600	14,700	16,000	19,200	21,300
C (,, Feb.) ...	3,000	7,700	9,700	13,200	17,300
D (,, April) ...	200	300	400	500	1,600

Soil leaching	Total hatch from 80 cysts after lapse of				
	30 days	40 days	50 days	60 days	70 days
A (unsterilized) ...	21,900	22,000			
B (sterilized Dec.) ...	22,100	22,500			
C (,, Feb.) ...	18,200	19,300	19,500		
D (,, April) ...	3,500	12,800	17,200	18,000	18,800

From the table and graph it will be seen that there is again a very definite lapse of time before a hatch of any magnitude commences in the case of the cysts in the soil leaching from the very recently (April) sterilized soil. Leaching from the soil sterilized in February produced only a slight retardation of hatching, and there was no retardation whatsoever in the leaching from soil sterilized in December. The retardation of hatching caused by very recent sterilization of the soil is proved, but the effect of sterilization is evidently not always so long lived as might appear from the previous paper.



Rate of hatching from *Heterodera schachtii* cysts in sterilized soil.

Pot Experiments relating to Effect of Soil Sterilization and Delayed Eelworm Infection.

These experiments were laid out (in 1936) in three series, A, B and C, and were carried out in exactly the same manner as those described in the previous paper. A sprouted tuber was planted in each pot (except pots 29, 30, 31, 32 which were planted on 20th May) on 7th April. Table II gives a summary of the lay out of the experiments.

Table III shows the yield (in ounces) from the pots in this experiment (weighings were made on 14th September) and the yields are also represented graphically by means of columns.

TABLE II.

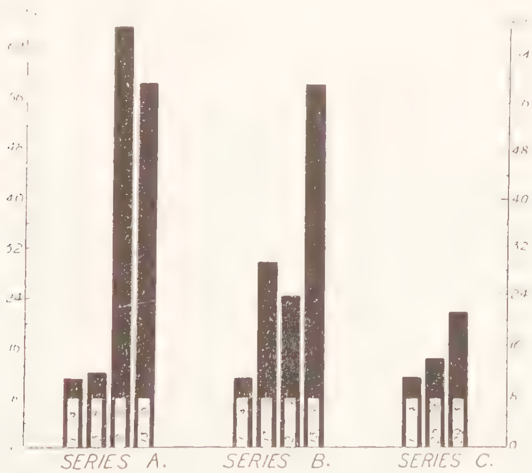
Series	Serial No. of pots	Treatment
A—Using potato sick soil	1, 2, 3, 4	Untreated, as obtained from field, containing 2 cysts per cc. (Controls)
	5, 6, 7, 8	Sterilized 1st April ; from 20th April (13 days after planting) larvae hatched out in the laboratory from 12,000 cysts (= 3 cysts per cc. of soil in pot) watered into each pot
	9, 10, 11, 12	Sterilized 1st April ; from 22nd May (when stalks were about 5 or 6 ins. high) larvae hatched out in the laboratory from 12,000 cysts watered into each pot
	13, 14, 15, 16	Sterilized 1st April ; no larvae added (Controls)
B—Using potato sick soil	1, 2, 3, 4,	Untreated (same pots as Series A) (Controls)
	17, 18, 19, 20	Sterilized 1st April ; afterwards infected with 12,000 cysts per pot (= 3 cysts per cc. of soil) ; during growing period plants watered with potato root excretion (obtained as leaching from pots of unsterilized soil growing potatoes)
	21, 22, 23, 24	Sterilized 1st April ; afterwards infected with 12,000 cysts per pot ; during growing period plants watered with water
	13, 14, 15, 16	Sterilized 1st April ; no cysts added (same pots as Series A) (Controls)
C—Using potato sick soil	1, 2, 3, 4	Untreated (same pots as Series A) (Controls)
	25, 26, 27, 28	Untreated ; tubers planted on 7th April ; plants completely removed from soil on 20th May and new tubers replanted on that date
	29, 30, 31, 32	Untreated ; tubers planted in these pots for first time on 20th May

TABLE III

Series	Serial No. of pots	Total yield of 4 pots in ounces
A	1, 2, 3, 4	11
	5, 6, 7. 8	12
	9, 10, 11, 12	67½
	13, 14, 15, 16	58½
B	1, 2, 3, 4	11
	17, 18, 19, 20	30
	21, 22, 23, 24	24½
	13, 14, 15, 16	58½
C	1, 2, 3, 4	11
	25, 26, 27, 28	14
	29, 30, 31, 32	22

OBSERVATIONS ON POT EXPERIMENTS.

All the plants in these experiments were sprayed three times during the growing season as a preventive against potato blight with the result that blight did not attack the plants.



Series A.

Pots 1, 2, 3, 4 :—

The plants in these pots remained very poor and exhibited typical severe overground symptoms of potato sickness.—Total yield—11 ozs.

Pots 5, 6, 7, 8 :—

As shown in Table II, larvae, hatching out in the laboratory from 12,000 cysts, were watered into these pots every second or third day from 20th April (on which date sprouts were just coming through the ground) onwards for about three weeks. The plants in these pots remained very poor and exhibited typical severe overground symptoms of potato sickness.—Total yield=12 ozs. (which was practically the same as pots 1, 2, 3, 4).

Pots 9, 10, 11, 12 :—

As shown in Table II, larvae, hatching out in the laboratory from 12,000 cysts, were watered into these pots every second or third day from 22nd May (when the plants were about five or six inches high) onwards for about three weeks. The plants in these pots grew exceptionally well during the entire growing period. They exhibited no overground symptoms of potato sickness but when lifted cysts were found to be numerous on the roots.—Total yield=$67\frac{1}{2}$ ozs. (which was in fact higher than yield from control uninfected pots 13, 14, 15, 16).

Pots 13, 14, 15, 16 :—

The plants in these pots were very good during the entire growing season. —Total yield=$58\frac{1}{2}$ ozs.

The above notes and table of yields for the plants in this Series show conclusively that when potato plants make some growth and develop a good root system before they are attacked by eelworm the parasite has little, or no, effect on subsequent growth or yield of the plants. It is also clear that even on very recently sterilized soil early infection with eelworm will produce typical severe potato sickness.

Series B.

This Series was laid down in order to verify or disprove an assumption put forward by the authors in the previous paper. In that paper it was suggested that the factor which caused the retardation of hatching of eelworm eggs in recently sterilized soil growing potatoes might be negatived by the addition to that soil of potato root excretion obtained from potatoes growing in unsterilized soil.

Pots 1, 2, 3, 4 :—See Series A.

Pots 17, 18, 19, 20 :—

These were watered every second or third day during the earlier period of growth with leachings from unsterilized soil growing potatoes (potato root excretion). The plants in these pots grew fairly well and remained fairly vigorous during the growing period. They did show overground symptoms of potato sickness but these were not very severe. Total yield=30 ozs. (which was higher than yield from pots 21, 22, 23, 24).

Pots 21, 22, 23, 24 :—

Watered with water only. Same remarks apply to the plants in these pots as to those in pots, 17, 18, 19, 20. Total yield=$24\frac{1}{2}$ ozs.

Pots 13, 14, 15, 16 :—See Series A.

The above notes and table of yields for the plants in this Series show that the addition of large quantities of potato root extract (obtained from plants growing in unsterilized soil) to pots 17, 18, 19, 20 did not negative the factor which produces a retardation of hatching. This is obvious from the fact that the plants in these pots were, if anything, better than the plants in pots 21, 22, 23, 24 where the retarding factor was known (from previous experiments) to be operative. Therefore, it is apparent that the assumption put forward in the previous paper in connection with this point has not been proved.

It may be noted in this Series that the plants growing in pots 21, 22, 23, 24 (recently sterilized soil afterwards infected with cysts) while very much better than the plants in pots 1, 2, 3, 4 (unsterilized potato sick soil) were nevertheless much poorer than plants in pots 13, 14, 15, 16 (recently sterilized soil not afterwards infected). Such a pronounced difference is not usually apparent but is easily explainable. It might, for example be due to the fact that the retardation of hatching of the eelworm eggs was not so long as usual or that the roots of the plants had not made vigorous growth before hatching of the eelworm larvae did commence.

Series C.

Pots 1, 2, 3, 4 :—See Series A.

Pots 25, 26, 27, 28 :—

The plants growing from the first tubers were exhibiting typical symptoms of potato sickness at the time they were removed. The plants

which grew from the second tubers exhibited severe symptoms of potato sickness soon after appearing above ground, and remained very poor during the period of their growth. The total yield from these plants was 14 ozs. (only slightly higher than the yield from the control plants 1, 2, 3, 4).

Pots 29, 30, 31, 32 :—

The plants in these pots did develop typical symptoms of potato sickness but these were not quite so severe as in pots 1, 2, 3, 4 and 25, 26, 27, 28 and the total yield was 22 ozs.

This Series, in the authors' opinion, brings forth a number of interesting points. One should have expected that by the time the first plants were removed from pots 25, 26, 27, 28 practically all the eelworm eggs in the soil would have been hatched and therefore that the second plants would be infected with eelworm only to a slight extent (by late hatching larvae). On the contrary the second plants exhibited severe symptoms of potato sickness which demonstrated that their roots were invaded by a sufficient number of larvae to produce the sickness. These larvae hatched subsequent to 20th May although potatoes had been planted in the soil on 7th April. The stimulation to hatch existed of course from the root excretion of the first plants and therefore hatching was taking place even before the second plants were sending out their roots. This fact, in the authors' opinion, explains why the second plants showed such severe symptoms of potato sickness. It is fairly certain that only a relatively small number of larvae entered the roots of these plants, but, as the roots were attacked at such an early stage of their development just as severe an effect was produced as if a much larger number of larvae had entered the roots at a more advanced stage of development.

The conditions of the plants in pots 29, 30, 31, 32 was such as to confirm an observation which the authors had casually made in the field on a few occasions. The observation was that when planting of potatoes on potato-sick soil was postponed until the season was well advanced (say in May) the degree of potato sickness which manifested itself was less severe than when potatoes were planted at a usual earlier date. Doubtless, this could be explained by assuming that in the warmer soil at the later date the roots grow much more rapidly and therefore are fairly well developed before hatching of the eelworm eggs in the soil commences.

Experiments Designed to Test the Possibility of Trap Cropping with Potatoes in Order to Reduce the Potential Eelworm Population of the Soil.

One often hears mentioned that trap cropping with potatoés might be a possible means of reducing the eelworm population of potato sick soil. Those who suggest this possibility generally argue that if potatoes are planted as a trap crop these potatoes will stimulate hatching of eelworm eggs in the soil and as a consequence their roots will become infested with eelworms. If such plants were then completely removed from the soil (roots included) while they are still fairly young (before the stage when cysts on the roots would fall off easily) it should follow that the soil would be freed to a very great extent (if not almost entirely) of eelworms and therefore would no longer be potato sick.

In setting out to design experiments on trap cropping with potatoes the authors determined to conform as closely as possible with what could be done in the field. If trap cropping should become a practical proposition in the field it would mean that during at least the first six or seven weeks of the growing season (say April and half of May) the ground would be given over to the trap crop. That would mean that the crop planted immediately after removing the trap crop could not be put in before (at the earliest) the middle of May. Two alternative propositions then present themselves, namely :—to replant with potatoes (which might still although late give a satisfactory crop) or to plant with turnips or some other crop.

Pots 25, 26, 27, 28 in Series C of the pot experiments already described clearly demonstrate that the replanting of potatoes immediately after removing a trap crop of potatoes is not a practical proposition because potato sickness developed in the plants from the secondly sown tubers almost to as great a degree as if no trap crop had been planted. Bearing in mind the actual result obtained in this pot experiment and also the reasons suggested by the authors as to why such a result should be obtained, it may be definitely said that the possibility of reducing the intensity of potato sickness by this procedure can be ruled out.

At this stage of the work the authors cannot make any pronouncement as regards the results which might be obtained by adopting the procedure of putting in a trap crop of potatoes, then planting a crop of a different kind after removing the trap crop (or leaving the ground in bare fallow) and not replanting with potatoes until the following year or a subsequent

year. A start has been made with both pot and plot experiments designed to test the usefulness (or otherwise) of such a procedure. The pot experiment (commenced in 1936) includes :—

4 pots—tubers planted 7th April ; plants removed 20th May.
4 ,, ,, ,, ,, ,, 3rd June.
4 ,, ,, ,, ,, ,, 12th June.

and also suitable control pots. All these pots will be replanted with potatoes in 1937.

In addition to the above pot experiment small field plots have been laid down on similar lines.

Although no results are yet available from these experiments it may be mentioned beforehand that there are various reasons why it should not be surprising if satisfactory results were not obtained. In the first place it is obvious that if the potato plants have got to the stage of having mature eelworms in the roots, or newly formed cysts on the roots, before they are removed from the soil, the roots must be removed in their entirety and without knocking any cysts off them. If such is not done many cysts already developed will remain in the soil and accordingly the object aimed at by trap cropping would not be achieved. In the field it would probably be almost impossible to remove the roots completely from the soil, and if any cysts had already formed undoubtedly some of them would be knocked off during the digging out operation.

If on the other hand the plants were removed from the soil before they had advanced very far in growth it is then possible that the complete removal of the roots would not be so important, but against this there is the fact that at the time of the removal of the plants some of the eelworm eggs in the soil would still be unhatched. The stimulus to hatch may become so reduced after removal of the plants that some of these still unhatched eggs may remain unhatched, but viable, in the soil.

Because of all the above considerations, and others not mentioned, the authors set themselves to devise an experiment to ascertain whether trap-cropping could be practised by just removing from the soil the young potato plant and tuber (from which the plant grew) only and leaving the roots behind. It was recognised that in following this procedure the plants would have to be removed at an early stage of their growth so that the eelworms in the roots might be deprived of the possibility of completing their development (assuming that development would not proceed

in the roots remaining in the soil). There was of course the possibility that although the plant and tuber were removed, the roots might, for some time afterwards, remain in a condition suitable for the eelworms to undergo further development within them. It was further realised that if this practice was adopted in the field many eelworm eggs would most likely be still unhatched at the time when the plants were removed.

It was decided to use for this experiment sandy soil which was naturally free from eelworm infestation (obtained from a grass field which had never previously grown potatoes). Twelve pots (Nos. 33 to 44 inclusive) were potted up with this soil in the usual manner and a sprouted tuber was planted in each pot on 7th April. From 20th April (on which date the sprouts were just appearing above ground) until 1st May larvae, hatching out in the laboratory, from 12,000 cysts (for each pot) were watered into each pot every second or third day.

On 20th May (43 days after planting and 30 days after larvae were first added) the plants and tubers were removed from pots 33, 34, 35, 36.

On 3rd June (57 days after planting and 44 days after larvae were first added) the plants and tubers were removed from pots 37, 38, 39, 40.

On 12th June (66 days after planting and 53 days after larvae were first added) the plants and tubers were removed from pots 41, 42, 43, 44.

In addition to the above numbered pots there were also some pots included as controls. These pots had the same soil ; tubers were planted in them on 7th April and larvae were watered into them from 20th April onwards. By examination of the roots of the plants in these control pots on the three dates mentioned above (20th May, 3rd June and 12th June), when plants and tubers were removed from the numbered pots, it was not possible on 20th May to find any trace of cysts, or females breaking through the epidermis. On 3rd June it was possible to see, by careful examination under a binocular microscope, some females just bursting through the epidermis. On 12th June small white cysts were abundant.

The numbered pots were allowed to stand in the open until 7th July on which date they were turned out and the roots examined. After a lengthy examination no cysts could be found on the roots in pots 33, 34, 35, 36. On the roots in pots 37, 38, 39, 40 some very small brown cysts were seen and on the roots in pots 41, 42, 43, 44 a greater number of somewhat larger brown cysts were seen. Subsequently (in October) the cyst content of the soil from each of these three lots of pots was determined.

This was done by taking five 20 grm. samples from each portion of soil. The results obtained were as follows :—

Pots 33, 34, 35, 36—no cysts in soil.

Pots 37, 38, 39, 40—approximately 0·5 cysts per cc. of soil having an average of 40 eggs per cyst (=20 eelworm eggs per cc. of soil).

Pots 41, 42, 43, 44—approximately 1·4 cysts per cc. of soil having an average of 87 eggs per cyst (=121 eelworm eggs per cc. of soil).

In determining the egg content of the cysts the eggs in all the cysts from each sample of soil were counted.

Some interesting facts have emerged from this experiment. It has been demonstrated that the development of eelworms (or cysts already formed) in the roots remaining in the soil after the removal of the potato plant and tuber is not immediately arrested but continues to proceed for some time. It is, however, evident from the observations made that development does not continue to proceed for very long after the removal of the plant. The roots of the four plants removed on 20th May had been infested with eelworms for approximately 30 days and therefore some of the female eelworms in these roots should have been nearly fully grown and ready for fertilisation but yet no cysts (not even white ones) were subsequently formed. In the case where females were breaking through the epidermis of the roots when the plants were removed only very small brown cysts were subsequently formed and in the case where white cysts were present when the plants were removed the brown cysts subsequently formed were found to contain not more than half the average normal number of eggs.

This experiment, although it does not afford any definite evidence as to the extent to which trap cropping by removal of plant and tuber might be beneficial or practical, gives much useful information. It has been shown than when plant and tuber are removed 30 days after roots are first infected with eelworm larvae no cysts are formed on the roots left behind in the soil. This would mean that under natural conditions in the field plants might be allowed to grow before removal for 30 days after eelworm eggs in the soil commence to hatch, or about six weeks after tubers are planted (assuming that hatching of larvae commences about 12 days after tubers are planted) without any fear that cysts would be formed from the

larvae which hatched during the 30-day period. Possibly no eelworm infection would be left behind in the soil (resulting from development of larvae which hatched) even if the plants were allowed to grow until say about 35 days after hatching commenced. Pots 37, 38, 39, 40 demonstrate that if plants are removed 44 days after hatching of eelworm eggs in the soil commences (or eight weeks after tubers are planted), a small degree of eelworm infection (20 eggs per cc. of soil) remains in the soil as a consequence of the development of some larvae which hatched during the 44-day period. Pots 41, 42, 43, 44 demonstrate that if plants are not removed until 53 days after hatching commences (66 days after tubers are planted) a fair degree of eelworm infection (121 eggs per cc. of soil in experiment) would be left in the soil as a consequence of the development of some larvae which hatched during the 53-day period.

From the experiment it is reasonable to expect that the degree of eelworm infestation in soil could be enormously reduced by putting in a trap crop of potatoes and removing the young potato plants and tubers (from which the plants grew) about six weeks after the tubers were planted. The degree of eelworm infestation still remaining in the soil would be only the number of eelworm eggs which were still unhatched at the time when the plants were lifted. If a second sowing of well sprouted tubers was made at the time when the first lot of potato plants was being removed the young plants growing from these tubers and the tubers themselves could be removed in about another three or four weeks and by that time the soil should be practically free from eelworm infestation.

As a result of their observations the authors are definitely of the opinion that if trap cropping with potatoes should ever become practicable as a control measure for potato eelworm it could only be done on the lines indicated in the above experiment. The tubers of the trap crop could be quickly planted in lines on the flat ground by dropping them into rather shallow holes. The young plants could be pulled out by hand without any digging and possibly in the great majority of cases the tuber would come up with the plant. In cases where the tuber did not come up it could be very easily removed with a light spade carried by the operator.

It is even possible that this method of trap cropping could be practised while the soil is devoted to the growing of another crop sown in drills (such as turnips, mangels, cabbage, etc.). The potato tubers could be planted between the drills of such a crop and the young potato plants pulled out at the correct time just like ordinary weeds. The whole

question of the possibility and usefulness of trap cropping with potatoes in this fashion is certainly worthy of further research.

Incidental Observations on the Hatching of Eelworm Eggs in the Laboratory.

During the course of hatching experiments in the laboratory the two following observations were made :—

During a particular period when work was being done on the water pipes of the College there was a considerable amount of iron oxide in the tap water. It was found that if this water was used for watering potatoes growing in pots of unsterilized soil the leaching obtained from these pots, although it contained potato root extract, did not stimulate the hatching of eelworm eggs. The inference drawn from this observation was that the iron oxide in the water had a definitely inhibiting effect on hatching and that the inhibiting effect was sufficiently potent to negative the stimulating effect of potato root excretion. The authors understand that an observation of a somewhat similar nature has already been made by Hurst and Triffitt and that it led to the experiments carried out by these workers in which various iron compounds were used.

It was also found that water taken from a particular pool into which a ditch opened possessed the power of stimulating the hatching of eelworm eggs almost to as great a degree as potato root excretion (leaching from pots of unsterilized soil growing potatoes). At first it was not clear why the water taken from the pool should possess this power but later it was discovered that drainage from potato fields not too far distant could possibly get into the ditch which fed the pool. The inference drawn was that the drainage contained a sufficient amount of potato root excretion to stimulate hatching of the eggs.

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An outbreak of Parasitic Necrosis in Turkeys caused by *Plagiorchis laricola* (Skrjabin).

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A turkey poult sent for post-mortem examination to the Veterinary Research Division, Ministry of Agriculture, Northern Ireland, was found to be suffering from a very heavy infection of a small fluke.

This fluke has been identified provisionally as *Plagiorchis laricola.*

There was a well-developed necrotic enteritis affecting the whole of the small intestine and duodenum. The intestine contained several hundreds of specimens of the parasite and examination of the faeces showed large numbers of fluke eggs.

A visit to the affected farm disclosed that the bird examined was the eleventh in a flock of twenty-three to die. All these birds showed similar symptoms—drooping, failure to put on weight and ultimately death. Another bird was examined and post-mortem results were similar to those in the first bird. Treatment with Kamala, 10 grains per bird was recommended.

This treatment was entirely unsuccessful as the remainder of the flock, with one exception, died.

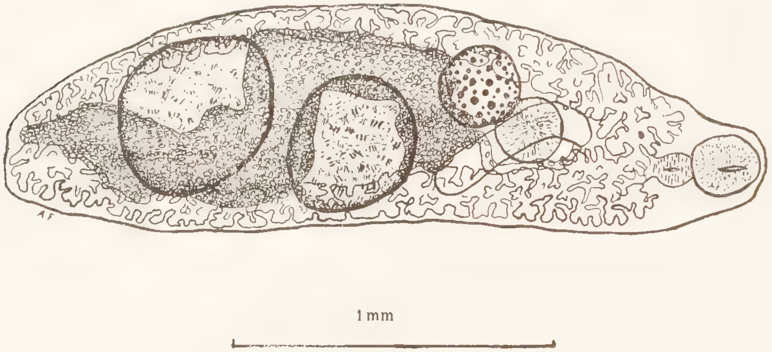
These turkey poults were grazed in a field, the lower end of which is continually water-logged and overgrown by common reed grass (*Arundo Phragmitis*). On the other side of the belt of reed grass is an extensive lake. These conditions would favour visits by the terns and gulls in which this parasite was first found by Skrjabin.

Description of Species.

The body is 2·39 mm. long by 0·70 mm. at its broadest point in the region of the anterior testicle. The body is leaf-like in outline, with the broadest point in the posterior half. The oral sucker is 0·17 mm. $\times$ 0·19 mm. The ventral sucker is 0·17 mm. in diameter. The pharynx is 0·11 mm. $\times$ 0·10 mm. The ventral sucker is 0·79 mm. behind the anterior extremity of the body. The ovary lies to the left of the ventral sucker behind its middle line. The margin of the ovary overlaps that of the

ventral sucker. The ovary is smooth and round with a diameter of 0·17 mm. The testes are tandem and are separated from each other and from the ovary by coils of the uterus. The anterior testicle is smaller than the posterior and measures 0·44 mm. × 0·44 mm. The posterior testicle measures 0·49 mm. × 0·47 mm.

The vitelline glands consist of closely compact follicules and extend from the level of the hind end of the oral sucker to the posterior end of the body. The glands of the right and left sides join in the region between the pharynx and the cirrus sac, but are separated at the posterior end by the uterus. The uterus extends from behind the ventral sucker to within a short distance of the posterior end of the body. The eggs measure 0·028 mm. × 0·019 mm.



Plagiorchis laricola (Skrjabin).

The cirrus sac is curved in an arc the posterior portion being straight. It is 0·49 mm. long. The posterior end is on a level with the posterior border of the ovary. It passes across the centre of the ventral sucker and curves round to the genital atrium in the middle line.

This is believed to be the first record of *Plagiorchis* in Northern Ireland.

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Journal of Helminthology, Vol. xv, *No.* 1, *January,* 1937, *pp.* 37–40.

Cysticercosis bovis and its prevention.

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In Australia this disease has occurred in many oxen on sewage farms and despite the economic loss involved, the farms have been prevented from raising cattle for human consumption. It has been claimed that this policy was adopted because of the risk to man of infestation with *Taenia saginata.* The object of this paper is to show that this economic waste is quite unnecessary, for cattle can be readily immunized against the disease so that there is no risk of *Taenia saginata* infestation in man from eating beef from such cattle.

In addition to being free from living cysticerci, beef should, however, if possible, be free from their old gritty degenerated remains and even from the small points of scar tissue which are sometimes left when the bladder worms degenerate. The eating of beef with such residual signs only of measles is, however, without risk to man. Such beef should no more be declared measly than a man showing pitting of the skin should be alleged to be suffering from small pox.

Method of Immunization.

To immunize cattle artificially against *Cysticercus bovis* we have given them large doses of fresh *Taenia saginata* eggs by mouth, usually 400,000 in number. In this way a primary infestation has been produced. When after waiting for 54 weeks to 23 months we have attempted to produce a secondary infestation by giving again the large dose of fresh eggs, we have invariably failed. The cattle were in fact immune and could not take a second infestation.

We have found further that immunity can likewise be produced by a naturally occurring primary infestation. In this case the animals have been allowed to feed on a sewage farm and so contracted very light natural infestation. Such infestations we have found able to produce effective immunity.

Of 88 cattle bought on the open market in Melbourne, which we attempted artificially to infest, each one contracted the disease. We have found that in such cases innumerable cysts developed up to approximately 30,000 in number. The bladder worms die after some time, when the cysts degenerate, diminish in size, leaving finally small dirty yellow, gritty, residual masses or, alternately, points of scar tissue. These gritty degenerated cysts we have never observed in the first three months after artificial infestation. Death of the worm, with degeneration of the cyst, may occur in the early stages of the disease, beef measles. In this case the cyst is filled with a greenish pasty material, but the dry, gritty, dirty yellow degenerated type of cyst we have never found in the first three months of the disease. In heavily infested oxen we have never found a living bladder worm at a later date than nine months after infestation of the ox. We believe, therefore, that the risk of infecting man does not exist for more than nine months after a heavy artificial infestation, but unfortunately we cannot at the moment give a precise date after which no gritty degenerated cysts can be found in the meat. We have found a few such cysts in one of three oxen two years and two months after the artificial infestation occurred, so that it seems not unlikely that 2½ or 3 years might have to elapse after primary infestation before we could guarantee that no sign of the former presence of the disease would exist in 100 per cent. of animals. Our researches on this aspect of the work are, however, not yet complete.

We have already published the first and second experiments on immunity in the Medical Journal of Australia (March 28, 1936, p. 417), and we also refer to the matter in the Annual Report of the Institute (August, 1935, p. 18).

In respect of the natural process of immunizing, a few remarks may be made. We have found that the eggs of the tapeworm tend to accumulate in the pasture round the sewage outlets. Consequently we would recommend to those who propose to immunize cattle naturally on sewage farms that they should depasture young calves in close proximity to the sewage outlets. Provided the sewage is sufficiently contaminated, this would ensure an early infestation and consequently an early immunity. Subsequently such calves could be pastured anywhere on the sewage farms with impunity, but if such animals were not slaughtered until they were about three years of age, we are satisfied that they could not spread Taeniasis saginata.

If cattle are taken on to sewage farms and allowed to graze anywhere, they may be on the sewage farm two years perhaps before they become infested and consequently even if they are killed at four years of age they may show a few cysts with the gritty degeneration of which we have already spoken.

This natural method of immunity would be quite effective and easily used in Syria and in certain parts of India and Africa, where the disease is very prevalent. It would not be very effective in those cities where there are very few inhabitants with *Taenia saginata*. In Melbourne, for example, we have found cattle, that have been on the sewage farm for six months, have shown 46% of infested animals. Since then we have located and treated 100 cases of tapeworm infestation in man and it seems not unlikely that this clearing out of the human cases may make the method of natural immunity inapplicable here. It is, however, impossible for us to say what number of residual cases exist in Melbourne, so that if the Government should ever consent to allow the raising of cattle for human consumption on the Melbourne sewage farm, it seems to us that the method of artificial infestation should be employed.

As to the minimum number of cysts which will produce immunity, we have not yet attempted by using falling doses of fresh *Taenia saginata* eggs to find the minimum number sufficient for this purpose, but we know that the naturally infested animals that have been depastured on the Melbourne sewage farm have acquired very complete immunity from only small infestations.

Dr. Gilruth examined ten oxen that had been on the Farm for an average of nine months. They showed in the sites of election amongst them 29 cysticerci. The rest of the dressed carcases were examined very carefully by the use of a bacon slicer, when 22 further cysts were found, all degenerated, that is to say, in the complete carcases only 51 in all, giving an average of five cysts to the carcase. If in nine months we only get an average of five cysts per animal, we conclude that a relatively small number of cysts would be quite sufficient to produce a good immunity. A summary of our immunity results is set out in the following paragraphs. It seems to us to demonstrate that the present waste of sewage irrigated farm lands is quite uncalled for.

Results of Experiments.

Seven oxen received an initial infestation of *Cysticercosis bovis* by feeding to each 400,000 *Taenia saginata* eggs given fresh, in a watery suspension. At the same time, by way of control, 24 oxen were fed with eggs from the same batch and all became infested. An attempt was then made to give the seven experimental animals a secondary infestation by again feeding the same number of eggs after a variable interval of time. Thus, two were re-fed after 54 weeks, two after 70 weeks and three after 23 months. The viability of these three batches of eggs was tested at the time by feeding to one, two and two oxen respectively, all of which became infested.

The post-mortem findings indicated that none of the seven experimental oxen had become infested as a result of the repeated dose of eggs. The two re-fed after 54 weeks showed a few small degenerated cysticerci, dry, gritty and of a dirty yellow colour, which in our opinion were derived from the primary infestation. The two oxen re-fed after 70 weeks showed almost complete recovery from the primary infestation. Of the three re-fed after 23 months, two showed no sign of the primary infestation, but the third still showed six degenerated cysts of the primary infestation, 1·5 mm. being the widest diameter.

In another experiment 20 oxen were grazed on a sewage farm, known to be contaminated with *Taenia saginata* eggs, over a period of not less than four years. Of 641 control oxen grazed on the same farm for only six months out of the four years, 46% showed infestation at post-mortem examination. These 20 oxen were drenched with 400,000 eggs each, the viability of the eggs having been controlled, yet none of them showed any sign of the secondary infestation at post-mortem. In fact, only two showed any sign of the primary infestation, having three small, degenerate cysts between them. The assumption that every animal grazed on this farm would be, or would have been, infested is proved by the resistance shown to secondary infestation in these 20 oxen.

Incidentally, we have found that eggs kept in saline in a refrigerator at about +2°C. die fairly quickly. It is important therefore to use eggs during the first month after collection. In an experimental attempt to infest oxen from the same batch of eggs but at various ages, it was found that no infestation occurred after the eggs had been 16½ weeks in saline in a refrigerator. In the immunity experiments recorded above, the eggs were used when only two days old.

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Taenia saginata : Its growth and propagation.

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INTRODUCTION.

WHEN the beef measle bladder worm is ingested by man it is believed that the head attaches itself to the mucosa of the jejunum, the caudal bladder disintegrates and segments develop in the neck region immediately behind the head at the rate of about 6–12 per day. Thus, segments immediately behind the head one day are pushed away from the proximity of the head by the formation of new segments each day. While a segment is being pushed further from the head it increases in size and alters its shape ; its sex organs, both male and female, develop and perform their functions. About 80 days are required for a segment to become ripe or for a bladder worm to develop into a complete tapeworm. The ripe posterior segments are detached almost daily. These are replaced by the formation of a similar number immediately behind the head. In this way a worm once fully grown maintains approximately the same length.

LENGTH AND COLOUR OF *Taenia saginata*.

We have obtained 200 specimens of *Taenia saginata*, but not all were complete. With the exception of one, all the worms examined were dead, some being in a contracted condition and others relaxed. Of those which were contracted, the shortest worm was 9 feet in length and the longest 20 feet. Of the relaxed worms, the longest, containing 950 segments, was 32 feet, and the shortest, consisting of 700 segments, was 15 feet. The live worm, when extended, measured 32 feet, but when touched, it contracted down to a length of 10 feet. Descriptions of inordinately long

worms (measuring 40–800 yards) are found in the literature. It is now generally believed that these mis-statements are due to the measurement of a number of worms obtained from cases of unrecognised multiple infestation.

The width and thickness of the worms we examined varied greatly. Usually the main body of the worm was a light cream colour, while the mature segments were not infrequently stained a deep yellow, especially near the edges. Two of the worms we obtained, however, had a grey pigmentation throughout the whole length. These were both obtained from one patient. Individual segments obtained from this patient over a period of months were always of a grey colour, but whether this was due to a particular variety of worm or to a persistent peculiarity of the intestinal contents of the patient, we have not been able to determine. Five other infested persons dining at the same table had worms of the usual light cream colour. We have never seen any green colouration such as is mentioned by Leuckart (1886) as occurring sometimes along the border of segments.

Rate of Growth.

We consider the average rate of growth of *Taenia saginata* is approximately 8–9 segments per day. A small number may grow more rapidly than this and some more slowly. If a very large number of worms (e.g., 31) is harboured by one patient, the rate of growth is apparently greatly reduced. Several of our patients, who later, on treatment, were shown to have one worm each, collected their segments for a fortnight, and the count of these gave an average of eight segments per day. A patient whom we treated unsuccessfully and from whom we obtained a length of worm consisting of 740 segments began to expel segments again 84 days after treatment. If we assume that the worm obtained its pre-treatment length before segments were detached, the rate of growth averaged about 9 segments per day. Leuckart believed that the expulsion of ripe segments did not keep pace with the development of new ones and consequently the worm increased in length with age until a large length was spontaneously discharged. If this view be true, then the number of segments in a mature worm must vary rhythmically. A small number of our cases has shown this spontaneous discharge of long lengths

of worm at more or less regular intervals of three to six months. However, in our cases it has been the exception rather than the rule. Leuckart considered that 14 segments were produced daily, but only 7–12 were expelled. If this is so, the number of ripe segments passed when a worm is mature does not indicate the full rate of growth. However, we know of infested persons who have passed detached segments only almost daily for periods of years and who, on being treated, were shown to harbour worms consisting of the average number of segments, *viz.*, approximately 900. Schimper suggested that the rate of growth varied according to the maturity of the worm, partly grown worms increasing more rapidly than fully grown ones.

Oliver fed measly beef to a Modammedan and a Hindu, both of whom passed segments for the first time 84 days later. The number of segments in the worms harboured was not recorded, but for worms of 800–900 segments, the rate of growth would have been approximately ten segments per day. A student of Perroncito swallowed a beef measle bladder worm and began to pass segments after 54 days. Fourteen days later a worm consisting of 866 segments was obtained from this patient. The growth in this case appears to have been extremely rapid, being probably at the rate of 16 segments per day. The possibility that the patient was harbouring a partially grown worm when the experiment was commenced must not be forgotten.

Schimper was of the opinion that the rate of growth varied with the diet, raw meat causing rapid growth. The Abyssinians, who eat raw beef, take an occasional dose of cousso to keep down the length of the worm. However, we have not found that the worms of the raw-meat-eating Syrians here have been any longer or that they detach more segments per day on an average than those of the infested Australians who do not make a habit of eating raw meat.

Number of Segments in a Complete Worm.

From our experience the number of segments in a complete worm is usually 800–900. The largest number we have counted has been 950. This worm was not broken and in accordance with our usual practice we counted the most anterior segments with the aid of a low power

microscope, so we are certain that all the segments of the worm were included in the count. The smallest complete worm examined contained only 700 segments. Leuckart, however, referred to worms containing as many as 1300 joints and Sommer fixed the number at about 1200.

Although there is some difference of opinion regarding the rate of growth and the number of segments in a worm, the question of clinical importance which depends on both is : How long must a patient be free from the expulsion of segments before it can be said that *Taenia saginata* is not present ? For practical purposes this has been estimated to be 100 days. It is possible for exceptions to occur, but we have not encountered any and are not aware of any in the literature.

Length of Life of *Taenia saginata*.

We believe that if *Taenia saginata* is not disturbed by treatment its length of life is usually limited only by the death of its host. This view goes a little further than that usually expressed by the statement that the parasite can live for many years. Amongst our Australian cases, who acquired their infestation in Victoria, who were harbouring only one worm when treated by us, and who had a history of practically uninterrupted passage of segments, were the following :—

Three infested for 20 years, one 18 years and one 14 years. Two of these cases had not previously been treated. Three had been treated during the first two years of their infestations. A long chain of segments was occasionally passed spontaneously, in which case the passage of segments was discontinued for a few weeks ; this suggests that only one worm was harboured at that time. Also the chance of acquiring more than one *Taenia saginata* in this State is small, as only two cases of multiple infestation were found amongst 48 patients who acquired the disease in Victoria (Penfold, Penfold & Phillips, 1936a). In the case of Syrians, there is a big chance of multiple infestation, especially while they are living in Syria, so that data relating to the length of life of worms harboured by them is not very reliable. However, the following eight patients had been infested for many years and harboured only one worm each when treated by us. They had not previously been treated and gave a history of the almost uninterrupted passage of segments.

2	infested	for	35	years.
1	,,	,,	30	,,
1	,,	,,	25	,,
3	,,	,,	20	,,
1	,,	,,	18	,,

Some of our Syrian cases, who had not been treated, were infested as long as 60 years, but as they harboured several worms, we cannot say that the first worm acquired was harboured throughout the period.

We do know of one case of apparently spontaneous cure. Nevertheless, we consider the above evidence is sufficient to suggest that the length of life of *Taenia saginata* if not disturbed by treatment is usually limited only by the death of the host.

The Number of Eggs in a Ripe Segment.

We have come to the conclusion that approximately 80,000 eggs per segment is the average, although the number varied from 5,000 to 130,000 in different specimens.

Our usual method of determining the number was the following :—

We took a chain of from 6–100 ripe segments from which we expelled the eggs, thoroughly mixed them in saline and counted the number in small volumes by means of a Fuchs-Rosenthal counting chamber 0·2 mm. in depth. A chain of segments was used so that we could be certain that no eggs had been naturally evacuated before we started expelling them. The chain was thoroughly washed in water to rid it of any adherent eggs from detached segments. The number of segments would be counted and the whole chain placed on a sheet of glass. A segment would be detached from the chain, held at its posterior end and stroked firmly several times from the posterior to the anterior end. The eggs would issue out of the narrow anterior end and were collected into saline by means of a Pasteur pipette. If any difficulty was experienced in getting the eggs out, the segment was divided longitudinally by ventro-dorsal incisions into three or four pieces, each of which was stroked as previously described. After this was repeated for all the segments, their remains and the glass plate were washed free of adherent

eggs with abundant saline. If the eggs in the saline were too dilute for accurate counting, they were concentrated. The volume of the counting chamber we used was $3\frac{1}{5}$ c.mm. Each batch of eggs was counted six times and the average struck. The counts were very uniform. In all probability a small percentage of eggs was left in some of the segments or their remains. However, microscopic examination of some of the apparently emptied segments showed that very few, if any, eggs were left. The following table gives to the nearest thousand the counts we obtained :—

Specimen No.	No. of segments in the chain from which the eggs were extracted and counted.	Average No. of Eggs per Segment.
1	50	95,000
2 Harboured by one patient.	50	99,000
3 Harboured by one patient.	50	104,000
4	100	105,000
5	50	80,000
6	50	74,000
7	6	5,000
8	20	23,000
9	150	89,000
10	70	71,000
11	50	105,000
12	20	96,000
13	30	11,000
14	50	120,000
15	35	86,000
16	80	70,000
17	30	9,000
18	40	130,000
19	50	130,000
20	50	80,000
21	20	49,000

Therefore, the average number of eggs in a segment of *Taenia saginata* is of the order of 80,000. The maximum number found was 130,000 and the minimum number 5,000.

Regarding variations in the number of eggs in individual ripe segments of the same worm, we have no absolute data, but from the appearance of the segments we consider the variations are small or at least most ripe segments from the same worm at the same time have a similar number of eggs.

We could approximately estimate the average number of eggs per segment from the appearance of the parasite. Those having a large number were coarse fat worms and those with a small number flat thin specimens.

We do not know the reason for the great differences in the number of eggs in segments of different specimens of *Taenia saginata*. It did not appear to us to be associated either with the type or with the volume of diet consumed by the host. Some well developed individuals with big appetites harboured only small worms and some miserable poorly-fed persons harboured large worms containing big numbers of eggs. The duration of infestation of the host also appeared to have no direct relationship.

Using the average figures of 80,000 eggs per segment and 9 detached segments a day, the number of eggs expelled by one worm in one day is 720,000. When one realises that these parasites can live for many years and are frequently allowed to do so by their hosts, the importance of offering a reward to induce patients to seek treatment is at once evident. The average duration of infestation of the first 83 of our cases was 13 years, and the average number of worms harboured was $2\frac{1}{2}$. Therefore, the average number of eggs expelled by each patient while infested, was probably $2\frac{1}{2} \times 720{,}000 \times 365 \times 13$, i.e., approximately 8,000 million.

Certainly most of the eggs in any natural circumstances are not ingested by cattle. Even of those ingested all do not produce cysticerci, because of acquired immunity in the cattle (Penfold, Penfold & Phillips, 1936b), but provided *Taenia saginata* infestation is not very frequent in the country concerned, the value of curing the patients and even paying them to be cured cannot be over-estimated. In countries such as Syria and Abyssinia, where the disease is very frequent, prevention of its spread could be most easily carried out by immunizing the cattle for a period of years.

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On some Lesions associated with Helminths in Birds of economic importance.

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Helminth parasites in small numbers infest a very large percentage of the birds of economic importance but the damage and loss for which they are responsible has never been calculated. In considering these parasites there are two schools of thought—the first considers every helminth as a possible pathological factor and cause of death. While our knowledge of avian diseases is still rather scanty this opinion is easily understood for birds often die showing lesions in the gut and the only obvious agent is the helminths present. Recently, however, opinion has swung in the opposite direction and given rise to a second school, in which there is a tendency to discount the dangers of helminthic infestation and to minimise their importance. In the Institute of Agricultural Parasitology some caution is always adopted at post-mortem examinations before diagnosing the cause of death as being definitely due to helminths for it is felt that there are many disease factors, imperfectly understood as yet, which probably have far reaching effects on their avian hosts. However, in certain cases pathological conditions have been noticed which were undoubtedly due to helminths.

In these cases about to be described it was generally not possible to observe the clinical signs of disease as the birds usually were sent in dead for post-mortem examination. The reports usually run that the bird was inactive and lazy and the feathers unkempt—a general condition of unthriftiness being apparent. In one case, however, a non-experimental guinea fowl which was kept at this Institute was noticed to be getting thin. There was loss of appetite, weakness and constipation which after two days gave way to a mucoid diarrhoea. Later still the stools became blood stained and the bird finally died ; the course of the disease lasting about eight days. Post-mortem examination revealed that the

bird had become emaciated and the gut showed a severe enteritis and inflammation, particularly in the duodenum and the first few inches of the small intestine. The contents were washed out and the gut wall examined under a binocular microscope. The mucous membrane was covered with petechial haemorrhages. The gut contents, which consisted largely of mucus, were heavily blood stained. Under low magnification many thousands of Ascarid larvae were seen—some free in the gut, some tangled in the mucus but the vast majority had burrowed their way into the mucosa causing haemorrhage and destruction of the intestinal glands. The bird was approximately a year old when it was purchased and regular faecal examinations during the four months it had lived at the Institute had shown it to be almost clean. From considerations of the helminthic fauna on the ground and from morphological evidence, it was concluded that these larvae were young forms of *Ascaridia lineata.* This worm occurs on several of the plots at the Institute and though certain stock turkeys are lightly infected, we have never before met with such a massive infestation. It is hardly possible to doubt that the presence of these larvae had been responsible for the death of this bird. It is worth pointing out too that this infection was purely accidental and picked up naturally by the bird from apparently lightly infected turkey plots on to which it had strayed.

With regard to the symptomology and pathology of *Ascaridia* infections, Ackert has remarked upon paralysis and inco-ordination as being produced in the host but no one seems to have recorded such a high degree of larval damage. No paralysis was noticed in this animal.

Capillaria spp. have from time to time been troublesome in chickens and turkeys. Recently three partridges came into the laboratory for examination and large numbers of *Capillaria longicollis* were recovered from all. This species has not been implicated as a pathological species up to the present. In the first case the bird showed extreme emaciation, weighing less than five ounces although full grown. The small intestine showed a severe enteritis, the gut wall being very thin for about 4 cms. From this short length alone 272 specimens of *Capillaria longicollis* were recovered. They had burrowed into the mucosa to such an extent that most of it had sloughed off and a copious haemorrhage had resulted. For a considerable distance the small intestine showed petechial haemorrhages.

Another partridge weighing $5\frac{1}{4}$ ounces, from the Norfolk district of England, contained 237 *C. longicollis* in the intestine. As before they were localised in a short region behind the duodenum and in this case they had actually ruptured the gut wall allowing contents to escape into the body cavity causing even more widespread inflammation than before. A third bird that came in contained 159 specimens and this too showed considerable reaction but the lesions were rather less pronounced.

In the light of the post-mortem findings in these three birds it does not seem unreasonable to incriminate this species as the producer of very serious injuries in the partridge and there is the distinct possibility that a similar reaction may be produced in birds of even greater economic importance. *C. longicollis* must therefore be added to *C. gallopavo*, *C. retusa* and *C. annulata* as of pathogenic significance.

Nodular formation in the caeca of birds due to the larvae of *Heterakis spp.* has also been noted by us recently. It is of frequent occurrence and may give rise to serious consequences. Such conditions have been noticed in partridges and pheasants and the species concerned is usually *H. isolonche.* The caecum becomes thickened with fibrous tissue with a diminution of the muscular activity. The caecum ceases to function. Finally the lumen becomes completely occluded and the contents become hard and cheesy. Such a condition seriously affects the well-being of the birds and may even cause death at times, and in others it has certainly hastened death. As the game industry is of some importance in this country, this genus should not be overlooked.

Finally *Davainea proglottina* has shown itself capable of producing reactions in gallinaceous birds. In a number of partridges and chickens that have come into the laboratory and which were found to contain this cestode—the scolices reaching many hundreds—the reaction in the gut consisted of enteritis and inflammation with considerable haemorrhage caused by the burrowing activities of these worms. About 1,000 scolices seems to be enough to produce a marked reaction in the partridge, rather more being necessary in the fowl.

Among other worms which have been found to be of significance as producers of lesions in birds is *Syngamus trachea.* This parasite is of wide distribution in this country and being able to attack a large variety of birds is of considerable economic importance. Attention is, however, drawn to a paper by the present writer in which nodular formation is

described in the trachea of the pheasant causing occlusion of the lumen and consequent dyspnoea and even asphyxiation.

Trichostrongylus tenuis is another parasite frequently met with causing marked lesions and considerable loss of many birds every year. These lesions will not be further described here but reference will be made to the Report of the Committee of Inquiry on Grouse Disease published in 1911, in which every aspect of the disease is described and discussed in full. It is important to notice, however, that as experimental transmissions to chickens have been effected in this laboratory several times, the domestic fowl is a potential host of this nematode.

It will be seen from these few personal experiences that helminths are important in the production of pathological lesions among birds.

Capillaria longicollis and *Ascaridia lineata*, which have not up to the present been considered as particularly pathogenic are here shown to be disease producers.

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On the Incidence of Abomasal Parasites in Fat Lambs from the same flock.

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Introduction.

A major difficulty in the diagnosis of parasitic gastro-enteritis of lambs is the uncertainty, under field conditions, of deciding on the degree of infestation necessary to cause disease. The clinical signs of worm-infestation are not always clear cut nor easily distinguishable from other causes of bad thriving in sheep. When post-mortem examination of a lamb is made, a certain number of worms are necessarily found since, except as the result of rigorous experimental precautions, all lambs contain some intestinal worms. Before diagnosis of death from worm infestation *per se* is justified there must therefore be some evidence that the dead lamb contained sufficient worms to cause its death. There must be evidence that the degree of infestation is beyond the normal range of variation. Otherwise to suppose the worms found at post-mortem to be the actual cause of death is an assumption.

The worm infestation of healthy lambs has been less studied than in those diseased. Cameron (1923), Morgan (1924) and Lewis (1930) were more particularly interested in species distribution and seasonal occurrence than in the numbers of any one parasite found in individual sheep. Robertson (1933) concluded that healthy lambs may contain 2,000–5,000 Ostertagia. Davey (1936) more recently has published figures for the numbers of *Ostertagia circumcincta, Haemonchus contortus* and *Trichostrongylus axei* present in the abomasums of fat lambs slaughtered in South-West Britain. This data, derived from slaughter-house material of uncertain origin, showed that a fat lamb may contain over 3,000 Ostertagia.

Management of Sheep.—In order to obtain further information on the abomasal parasites of healthy sheep, the abomasums from 75 fat lambs from the Duthie Experimental Stock Farm, Rowett Institute, were examined in detail. The lambs were from the Duthie Farm's commercial flock. The object of management of this flock, composed of Greyface ewes with Down cross lambs, is to obtain a high percentage of fat " milk " lambs—that is to say, fat lambs sold direct from ewe to butcher. The rams used were Oxford, Suffolk and Southdown, lambing took place in February and in March, lambs were sold fat from early May to mid-September. Weaning was on 13th August. All lambs were fed on ewes' milk, pasture and concentrates; the amount of concentrates fed being $\frac{1}{4}$–$\frac{1}{2}$ lb. daily. The lambs were *not* dosed for worms. The general system of management employed has already been described by Fraser & Middleton (1931). Since the lambs were from the Duthie Farm's commercial flock, commercial considerations had to be taken into account. Thus, owing to a period of low prices, no lambs were slaughtered during June. Neither were the lambs maintained under those strictly experimental conditions of management which permits finality in the interpretation of results.

Counting of worms.—The abomasal contents were obtained immediately after slaughter and preserved for examination. Ostertagia and Trichostrongylus were individually counted in duplicated aliquot samples of the diluted abomasal contents by the method previously described by us (1936). A complete count of Haemonchus was made.

Results.

The detailed figures of the worm-counts are shown in Table I (page 56). Date of slaughter, whether single or twin, and the numbers of Haemonchus, Ostertagia and Trichostrongylus are recorded for each lamb. Where stomach contents were lost the fact is mentioned.

Seasonal Incidence.—The average number of worms of each species found in lambs slaughtered in each of the four months, May, July, August, September, are shown in Table II (page 57).

Haemonchus.—The figures for Haemonchus infestation are most reliable since this worm is readily isolated from the diluted stomach contents and

was, moreover, individually counted. It is clear that in the flock investigated, infestation with Haemonchus was negligible until August. In August counts up to 354 worms were obtained. In September the figures showed a further increase, with one exceptionally heavy infestation of 2,100 worms.

Ostertagia.—Ostertagia infestation remained fairly steady from May to September. There was, for example, a heavy infestation of 3,200 worms in a lamb killed on 9th May. The differences between the average figures of lambs killed in each of the four months are of no practical importance.

Trichostrongylus axei.—All that can safely be said of the figures for this species is that the worm is present throughout the grazing season but is never present in great numbers. The individual counts are of doubtful accuracy since the worm is too small for complete individual counting and since the method of sampling employed is unsatisfactory for worms present in such relatively small numbers.

Degree of Infestation.—The lambs were slaughtered at an average live weight of 80–100 lbs. killing out at 40–50 lbs. dead. They were sold privately and killed for local trade, which requires a slightly heavier and definitely more finished carcase than the London trade. The carcase quality was rather above that common in the district.

Lambs killed in May were lambed in February ; those killed in July and later were lambed in March. The lambs killed in May were about three months of age ; those killed in July were between three and four months. In both cases the live weight increase was between ½ and 1 lb. per day. In every way the lambs appeared healthy.

Those lambs slaughtered on 9th May and on 1st July had thriven as well as could be expected under the conditions of management—which aimed at the economical production of fat lambs, and the degree of infestation of lambs which took longer to finish was not greatly different, apart from the late appearance of Haemonchus. It can therefore be stated, quite definitely, that a lamb can bear an infestation of 3,000–4,000 Ostertagia without showing any recognisable clinical symptoms. The question whether or not the lambs would have thriven even better had they been worm-free or had they had a less degree of infestation cannot be answered by the results of this investigation and is obviously one which requires experimental treatment for its solution.

TABLE I

No. of lamb	Date of slaughter	Whether single or twin	Number of Haemonchus	Number of Ostertagia	Number of *Trichostr. axei*
1	9th May, 1935 ...	—	0	1,920	60
2	9th May, 1935 ...	—	0	600	0
3	9th May, 1935 ...	—	1	3,200	0
4	9th May, 1935 ...	—	0	800	0
5	9th May, 1935 ...	—	0	220	0
6	12th May, 1935 ...	Single	0	890	0
7	12th May, 1935 ...	Twin	0	1,460	0
8	12th May, 1935 ...	Twin	0	1,780	0
9	12th May, 1935 ...	Twin	0	450	0
10	12th May, 1935 ...	Single	0	1,190	40
11	12th May, 1935 ...	Single	0	1,890	0
12	20th May, 1935 ...	?	0	170	0
13	20th May, 1935 ...	Twin	0	1,420	20
14	20th May, 1935 ...	Single	0	850	0
15	20th May, 1935 ...	Single	0	2,640	0
16	20th May, 1935 ...	Twin	0	1,500	60
17	20th May, 1935 ...		Stomach con	tents lost	
18	1st July, 1935 ...	Twin	0	1,250	20
19	1st July, 1935 ...	Twin	8	1,950	0
20	1st July, 1935 ...	Twin	5	2,480	0
21	1st July, 1935 ...	Twin	2	1,200	40
22	1st July, 1935 ...	Twin	1	1,560	0
23	1st July, 1935 ...		Stomach con	tents lost	
24	1st July, 1935 ...	Twin	5	920	20
25	1st July, 1935 ...	Twin	0	760	120
26	1st July, 1935 ...	Twin	0	1,870	20
27	1st July, 1935 ...	Twin	4	2,430	0
28	1st July, 1935 ...	Single	2	240	0
29	1st July, 1935 ...	Twin	3	1,360	0
30	4th July, 1935 ...	Single	1	190	60
31	4th July, 1935 ...	Single	1	170	0
32	4th July, 1935 ...	Single	0	100	40
33	4th July, 1935 ...	Twin	2	980	0
34	8th Aug, 1935 ...	Twin	0	1,780	40
35	8th Aug, 1935 ...	Twin	2	410	20
36	8th Aug., 1936 ...	Twin	54	1,330	40
37	8th Aug., 1935 ...	Twin	230	3,310	0
38	8th Aug., 1935 ...	Single	64	1,850	100
39	8th Aug., 1935 ...	Single	94	4,670	0
40	8th Aug., 1935 ...	Twin	41	1,430	40
41	8th Aug., 1935 ...		Stomach con	tents lost	
42	8th Aug., 1935 ...	Twin	187	3,620	20
43	8th Aug., 1935 ...	Twin	76	3,270	40
44	8th Aug., 1935 ...	Twin	57	1,770	40
45	8th Aug., 1935 ...	Single	51	1,050	0
46	15th Aug., 1935 ...	Twin	188	920	100
47	15th Aug., 1935 ...	Twin	1	490	20
48	15th Aug., 1935 ...		Stomach con	tents lost	
49	15th Aug., 1935 ...	Single	521	1,870	40
50	15th Aug., 1935 ...	Twin	54	690	60

TABLE I—*continued.*

No. of lamb	Date of slaughter	Whether single or twin	Number of Haemon-chus	Number of Oster-tagia	Number of *Trichostr. axei*
51	15th Aug., 1935 ...	Single	354	3,430	0
52	15th Aug., 1935 ...	Twin	128	490	0
53	15th Aug., 1935 ...	Single	32	150	80
54	15th Aug., 1935 ...	Twin	56	1,500	0
55	15th Aug., 1935 ...	Twin	259	1,840	120
56	15th Aug., 1935 ...	Single	214	1,130	0
57	15th Aug., 1935 ...	Single	30	190	20
58	15th Aug., 1935 ...	Twin	14	1,130	40
59	15th Aug., 1935 ...	Twin	2	620	40
60	15th Aug., 1935 ...	Twin	116	1,170	0
61	15th Aug., 1935 ...	Single	9	1,370	80
62	15th Aug., 1935 ...	Twin	141	4,470	0
63	26th Aug., 1935 ...	Twin	27	2,480	0
64	26th Aug., 1935 ...	Single	234	810	0
65	26th Aug., 1935 ...	Twin	60	490	0
66	26th Aug., 1935 ...	Single	144	250	0
67	26th Aug., 1935 ...	Single	12	2,080	0
68	26th Aug., 1935 ...	Single	29	540	0
69	26th Aug., 1935 ...	Twin	96	1,590	0
70	16th Sept., 1935	Twin	26	80	0
71	16th Sept., 1935	Twin	334	3,470	60
72	16th Sept., 1935	Twin	468	720	0
73	16th Sept., 1935	Single	54	1,330	0
74	16th Sept., 1935	Twin	31	190	0
75	16th Sept., 1935	Twin	2,100	2,610	0

TABLE II

Month	No. of lambs slaughtered	Haemon-chus	Oster-tagia	Tricho-strongylus
May	16	0	1,311	11
July	15	2	1,164	21
August	34	108	1,591	27
September	6	502	1,400	10

Relative Infestation of Single and Twin Lambs.—In view of their superior nutrition, it might reasonably be expected that under similar pastoral conditions the worm infestation of single lambs would be less than that of twins. Accordingly from 12th May onwards lambs were distinguished as single or twin before slaughter. There was some little dubiety about the lambs killed on 12th and 20th May since they were not marked

until marketing. All lambs born in March and killed from 1st July onwards were ear-marked as single or twin at birth and were thus more readily distinguished when slaughtered.

Taking all the records of single lambs and comparing them with those of twins, the average infestation of single lambs with Ostertagia was 1,233 ; of twins 1,582. The corresponding figures for Haemonchus were single lambs 83, twin lambs 81 (excluding No. 75). When all the individual worm counts are averaged there is thus no clear indication of a differential worm infestation of single and twin lambs.

When, however, the figures are arranged according to the period in which the lambs were killed, there is an indication of a much heavier infestation of twin lambs killed in July compared with that of single lambs killed in the same month. The figures of Ostertagia infestation in the different months are shown in Table III below :—

TABLE III

Average Ostertagia Infestation.

Month.	Singles	Twins
May	1,492	1,322
July	175	1,524
August	1,491	1,620
September	1,330	1,414

The individual counts for lambs killed in July are shown in Table IV :—

TABLE IV

Haemonchus and Ostertagia Infestation of Lambs killed in July.

Single Lambs		Twin Lambs	
Haemonchus	Ostertagia	Haemonchus	Ostertagia
2	240	0	1,250
1	190	8	1,950
1	170	5	2,480
0	100	2	1,200
		1	1,560
		5	920
		0	760
		0	1,870
		4	2,430
		3	1,360
		2	980

The figures shown in Table IV are extremely suggestive, but obviously require confirmation with a larger number of lambs kept under strictly controlled conditions. An attempt at such confirmation is projected and, until further results are secured it would be premature to speculate on the significance of those already secured.

Summary.

1. To determine the upper limit of the abomasal worm infestation of healthy lambs was the main object of the investigation. The results show that a lamb slaughtered fat before or shortly after weaning and therefore presumably a healthy lamb, may contain up to 2,100 Haemonchus and up to 4,670 Ostertagia.

2. Infestation with *Haemonchus contortus* is negligible until August.

3. Infestation with Ostertagia remains almost steady from early May until mid-September.

4. Infestation with *Trichostrongylus axei* occurs from May until mid-September, but is never a heavy one.

5. The evidence suggests, but does not prove, that in mid-summer there is a wide difference in the infestation of single and twin lambs.

6. The results, so far as they affect the seasonality of infestation are, strictly speaking, applicable only to the flock of the Duthie Experimental Stock Farm, but are probably true for the North-East area of Scotland.

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